UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION,
PESTICIDES
AND TOXIC
SUBSTANCES

February 3, 2006

MEMORANDUM

Subject: Efficacy Review for EPA Reg. No. 71847-2, Klor-Kleen;
DP Barcode: 325838

From: Tajah L. Blackburn, Ph.D., Microbiologist 2/3/06
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510C)

Thru: Nancy Whyte, Team Leader February 3, 2006
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510C)

To: Emily Mitchell PM 32/Wanda Henson
Regulatory Management Branch II
Antimicrobials Division (7510C)

Applicant: Medentech Ltd
Whitemill Industrial Estate, Clonard Road
Wexford, Ireland

Formulations from Label

Active Ingredient(s)	% by wt.
Sodium Dichloroisocyanurate.....	48.21%
Inert Ingredients.....	51.79
Total	100.00%

I BACKGROUND

The product, Klor-Kleen (EPA Reg. No. 71847-2), is a registered disinfectant (bactericide, tuberculocide, virucide) for use on hard, non-porous surfaces in institutional, animal care, and hospital or medical environments. Due to potential public health and socioeconomic impact, the Agency has requested that the applicant submit efficacy data for the following animal pathogens: Infectious Bursal Disease (Gumboro Disease), Transmissible Gastroenteritis virus, Swine Vesicular Disease, African Swine Fever, Classical Swine Fever (Hog Cholera), and Fowl Pox virus (Avipox). The current data package contains the efficacy studies, as requested.

This data package contains a letter from the Institute for Animal Health, Pirbright Laboratory, Formulation Data sheet, and five efficacy studies (No MRID Numbers were issued).

Note—The submitted efficacy studies were conducted at Quality Control Unit, Central Veterinary Laboratory, in Addlestone, Surrey. Based on the location of the testing, the efficacy studies were not formatted properly. The Agency is willing to overlook this deficiency considering the limited number of laboratories conducting these tests globally.

Note—This testing was conducted on brand names Aquasept and Septrivet 17. Confidential formulation data, to show product similarity, is enclosed.

II USE DIRECTIONS

The product is designed for use in disinfecting pre-cleaned, hard, non-porous surfaces such as floors. Directions on the proposed label provided the following information regarding preparation and use of the product as a disinfectant: Add 1 Klor-Kleen tablet to 1 gallon of water to obtain a concentration of ~1000 ppm of available chlorine. Apply the use solution to pre-cleaned, hard, non-porous surfaces. For sprayer applications use a coarse spray device. Spray use solution 6-8 inches from surface; rub with brush, sponge, or cloth. Treated surfaces must remain wet for 10 minutes. Remove product by wiping with a brush, sponge, or cloth.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Virucides

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a

specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. These Agency standards are presented in DIS/TSS-7.

Virucides – Novel Virus Protocol Standards

To ensure that a virus protocol has been adequately validated, data should be provided from at least 2 independent laboratories for each product tested (i.e., 2 product lots per laboratory).

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. "Assessment of Inactivation of Infectious Bursal Disease Virus (Gumboro Disease) by Aquasept" performed by M. Faithfull. Study conducted at Quality Control Unit, Central Veterinary Laboratory. Study completion date—January 8, 1999. Study number CVLS/95/98.

This study was conducted against Infectious Bursal Disease (IBDV; strain CS88/QCP 346) using SPF chicks. Five tubs of test material, Aquasept 200, were received. Each tub contained 320 tablets. The test material was diluted in WHO hard water by adding a tablet of Aquasept to 1 liter, 2 liters, or 3 liters to yield the following concentrations 1000, 500 and 333, respectively. The concentrations were prepared 30 minutes prior to use to allow sufficient time for full dissolution of the tablets. A suspension of IBDV was prepared by adding 1ml to 9 ml of 0.03% bovine albumin diluted in WHO hard water. **Separate 1 ml volumes of virus suspension were added to 1ml each of disinfectant concentration and held at 20°C while shaking for 10 minutes**. Serial tenfold dilutions of the virus suspension from were prepared by adding 0.5 ml to 4.5 ml of peptone broth (neutralizer) to give a range of dilutions from 10^{-1} to 10^{-6}. After 30 minutes at 20°C each of the five chicks were inoculated with one of the mixtures by dropping 0.1 ml onto the conjunctiva. Three days post inoculation, all chicks were killed and their Bursae of Fabricius were removed for both microscopic and macroscopic evaluation of lesion. Any bursae which did not demonstrate macroscopic lesions of IBD infection, was tested for the presence of IBDV using agar gel diffusion test according to the Qualitative agar gel precipitation test (to detect IBD antigens or antibodies). The median chick infective dose (CID_{50}) for the titration was calculated using the method of Reed & Muench.

Note—It was clear from macroscopic evaluation of the Bursae of Fabricius that the test was invalid, as a virus titer end point on the back titration was not obtained (greater than $10^{6.1}$ CID_{50}/0.1 ml). Therefore a repeat test was performed.

2. **"Assessment of Inactivation of Transmissible Gastroenteritis Virus by Aquasept" performed by CE Jenkins. Study conducted by Quality Control Unit, Central Veterinary Laboratory. Study completion date—May 3, 1999. Study number CVLS/96/98.**

The study was conducted against Transmissible Gastroenteritis virus (TGEV) using A72 cells. Five tubs of test material, Aquasept 200, were received. The test material was diluted in WHO hard water by adding a tablet of Aquasept to 1 liter, 2 liters, or 3 liters to yield the following concentrations 1000, 500 and 333, respectively. The concentrations were prepared 30 minutes prior to use to allow sufficient time for full dissolution of the tablets. The virus was suspended in WHO hard water by adding 1 ml of TGEV to 9 ml of water. A separate suspension of TGEV was made in WHO hard water containing 0.03% (w/v) bovine albumin, by adding 2 ml of virus to 18 ml of diluent. A suspension of IBDV was prepared by adding 1ml to 9 ml of 0.03% bovine albumin diluted in WHO hard water. **Separate 2.5 ml volumes of virus suspension were added to 2.5 ml each of disinfectant concentration and held at 20°C while shaking for 30 minutes followed by centrifugation for 2 minutes.** Serial tenfold dilutions were made from each mixture to give a range from neat to 10^{-10}. The first dilution from each mixture was made in culture medium containing 1% (w/v) milk powder for neutralization. Subsequent dilutions were made in cell culture. Each dilution was added to 8 wells of a 96-well microtiter plate containing A72 cell suspension. A separate column was used for each dilution and a separate plate for each mixture. The plates were sealed and incubated for five days at 37°C. At the end of the test period, the cells were examined microscopically for TGEV-specific cytopathic effects. Virus titers were expressed as the median tissue culture infective dose per volume inoculated.

3. **"Assessment of Inactivation of African Swine Fever Virus by Aquasept" performed by S. Williams (IAH, Pirbright). Study conducted by Quality Control Unit, Central Veterinary Laboratory. Study completion date—April 29, 1999. Study number—CVLS/99/98.**

The study was conducted against African Swine Fever virus (ASFV) using primary bone marrow cells. Five tubs of test material, Aquasept 200, were received. The test material was diluted in WHO hard water by adding a tablet of Aquasept to 1 liter, 2 liters, or 3 liters to yield the following concentrations 1000, 500 and 333, respectively. The concentrations were prepared 30 minutes prior to use to allow sufficient time for full dissolution of the tablets. A suspension of ASFV was prepared by adding 1ml to 9 ml of water. A separate suspension of ASFV was made in WHO hard water containing 0.03% bovine albumin (w/v), by adding 2 ml of virus to 18 ml of diluent. **Separate 2.5 ml volumes of virus suspension were added to 2.5 ml each of disinfectant concentration and held at 20°C while shaking for 30 minutes followed by centrifugation for 2 minutes.** Serial tenfold dilutions were made from each mixture to give a range from neat to 10^{-6}. The first dilution from each mixture was made in culture medium containing 1% (w/v) milk powder for neutralization. Subsequent dilutions were made in cell culture. A sample size of 300 µl of each dilution was dispensed into each of 3 separate tubes containing 1.5 ml of primary bone marrow cells. The tubes were incubated for five days at 37°, with examinations conducted on the third, fourth, fifth days post incubation for evidence of hemagglutination. On the fifth day, 0.1 ml of

pig red blood cells was added to each tube and incubated for an additional day. Virus titers were expressed as the median hemadsorbing units per ml.

4. "Assessment of Inactivation of Classical Swine Fever Virus by Aquasept" performed by CE Jenkins. Study conducted at by Quality Control Unit, Central Veterinary Laboratory. Study completion date—December 1, 1998. Study number—CVLS/101/98.

The study was conducted against Classical Swine Fever virus (CSFV) using PK-15 pig kidney cells. Five tubs of test material, Aquasept 200, were received. The test material was diluted in WHO hard water by adding a tablet of Aquasept to 1 liter, 2 liters, or 3 liters to yield the following concentrations 1000, 500 and 333, respectively. The concentrations were prepared 30 minutes prior to use to allow sufficient time for full dissolution of the tablets. A suspension of CSFV was prepared by adding 1ml to 9 ml of water. A separate suspension of CSFV was made in WHO hard water containing 0.03% bovine albumin (w/v), by adding 2 ml of virus to 18 ml of diluent. **Separate 2.5 ml volumes of virus suspension were added to 2.5 ml each of disinfectant concentration and held at 20°C while shaking for 30 minutes followed by centrifugation for 2 minutes.** Serial tenfold dilutions were made from each mixture to give a range from neat to 10^{-6}. The first dilution from each mixture was made in culture medium containing 1% (w/v) milk powder for neutralization. Subsequent dilutions were made in cell culture. Each dilution was added to 8 wells of a 96-well microtiter plate containing confluent PK-15 pig kidney cells. A separate column was used for each dilution and a separate plate for each mixture. The plates were sealed and incubated for four days at 37°C. At the end of the test period cells were fixed using two changes of 20% acetone, then drained and dried under a bench lamp. They were stained using a panpestivirus monoclonal antibody followed by a rabbit anti-mouse horseradish peroxidase conjugate and the staining visualized using a carbazole substrate. The stained plates were examined microscopically for CSFV-specific staining. Virus titers were expressed as the median tissue culture infective dose per volume inoculated.

5. "Assessment of Inactivation of Fowl Pox Virus (Avipox) by Aquasept" performed by CE Jenkins. Study conducted at Quality Control Unit, Central Veterinary Laboratory. Study completion date—March 9, 1999. Study number—CVLS/102/98.

The study was conducted against Fowl Pox Virus (FPV) using embryonating eggs. Five tubs of test material, Aquasept 200, were received. The test material was diluted in WHO hard water by adding a tablet of Aquasept to 1 liter, 2 liters, or 3 liters to yield the following concentrations 1000, 500 and 333, respectively. The concentrations were prepared 30 minutes prior to use to allow sufficient time for full dissolution of the tablets. A suspension of FPV was prepared by adding 1ml to 9 ml of water. A separate suspension of FPV was made in WHO hard water containing 0.03% bovine albumin (w/v), by adding 2 ml of virus to 18 ml of diluent. **Separate 2.5 ml volumes of virus suspension were added to 2.5 ml each of disinfectant concentration and held at 20°C while shaking for 30 minutes followed by centrifugation for 2 minutes**. Each Serial tenfold dilutions were made from each mixture using antibiotic broth, to give a range from neat to 10^{-6}. Each dilution (0.1 ml) was inoculated onto the chorioallantoic membrane (CAM) of 5 separate 10-day old embryonating chicken eggs. The eggs were

incubated for up to 7 days at 37°C, and inspected daily using a candling lamp. Eggs containing dead embryos within 24 hours were discarded as non-specific. Thereafter, any eggs containing dead embryos were retained at 4°C until the end of the incubation period, when all of the surviving embryos were chilled. Following incubation, the eggs were opened and CAMS examined for evidence of FPV infection. Virus titers were expressed as the median embryo infective dose per volume inoculated.

V RESULTS

A. Detection of IBDV in the bursae of chicks inoculated with which had been pre-treated with Aquasept disinfectant.

Concentration of Aquasept	Dilution of Mixture	Result	Titer (CID_{50})
1000 ppm	Neat	0/5*	
	10^{-1}	0/5	$<10^{0.5}$/0.1 ml
500 ppm	Neat	0/5	
	10^{-1}	0/5	
	10^{-2}	0/5	$<10^{0.5}$/0.1 ml
333 ppm	Neat	4/5	
	10^{-1}	2/5	
	10^{-2}	0/5	$10^{3.945}$/0.1 ml

The titer of the virus was shown to be $10^{4.625}$ CID_{50}/0.1 ml.

B. Assessment of Inactivation of Transmissible Gastroenteritis Virus by Aquasept

Titrated substance	Virus titer
TGEV + WHO hard water	$10^{4.125}$
TGEV + WHO hard water + albumin	$10^{4.25}$
TGEV + 1000 ppm Test Material	$\leq 10^{0.5}$
TGEV + 500 ppm Test Material	$\leq 10^{0.5}$
TGEV + 333 ppm Test Material	$\leq 10^{0.5}$

C. Assessment of Inactivation of African Swine Fever Virus By Aquasept

Titrated Substance	Virus titer
ASFV + WHO hard water	$10^{5.75}$
ASFV + WHO hard water + Albumin	$10^{5.75}$
ASFV + 1000 ppm Test Material	$\leq 10^{1.0}$
ASFV + 500 ppm Test Material	$\leq 10^{1.0}$
ASFV + 333 ppm Test Material	$\leq 10^{1.0}$

D. Assessment of Inactivation of Classical Swine Fever (Hog Cholera) Virus By Aquasept

Titrated Substance	Virus titer
CSFV + WHO hard water	$10^{5.0}$
CSFV + WHO hard water plus **yeast***	$10^{5.0}$
CSFV + 1000 ppm Test Material	$\leq 10^{0.5}$
CSFV + 500 ppm Test Material	$\leq 10^{0.5}$
CSFV + 333 ppm Test Material	$\leq 10^{0.5}$

* Note—The protocol makes no mention of yeast. Bovine albumin is used consistently during testing. The Agency believes that the mention of yeast in the result table is an error.

E. Assessment of Inactivation of Fowl Pox Virus By Aquasept

Titrated Substance	Virus titer
FPV + WHO hard water	$10^{3.17}$
FPV + WHO hard water + albumin	$10^{4.17}$
FPV + 1000 ppm Test Material	0
FPV + 500 ppm Test Material	0
FPV + 333 ppm Test Material	0

VI CONCLUSIONS

1. The proposed claims for disinfection at a concentration of 937 ppm of available chlorine (in the presence WHO hard water) for 10 minutes against **Infectious Bursal Disease (Gumboro Disease) Virus**, and for 30 minutes against **Transmissible Gastroenteritis Virus, Swine Vesicular Disease Virus**, **African Swine Fever Virus**, **Classical Swine Fever (Hog Cholera) Virus**, and **Fowl Pox Virus** have been demonstrated with submission of the requested efficacy data. Recoverable virus titers of at least 10^4 were achieved. Complete inactivation (no growth) was indicated at most dilutions tested. Cytotoxicity, if any, was not reported.

VII RECOMMENDATIONS

1. The proposed label must be modified for **Transmissible Gastroenteritis Virus, Swine Vesicular Disease Virus**, **African Swine Fever Virus**, **Classical Swine Fever**

(Hog Cholera) Virus, and **Fowl Pox Virus** to reflect the conditions in which efficacy was demonstrated. **The contact time of 10 minutes must be changed to 30 minutes, as this is consistent with the accepted efficacy studies.** Furthermore, re-application directions and/or submersion application should be included with the use directions of this product for disinfection of the animal pathogens. By amending the use directions (i.e. re-application and/or submersion), the user is ensured the necessary contact time needed to demonstrate efficacy.

2. The label claim against **Infectious Bursal Disease (Gumboro) virus** in the presence of **937-ppm available chlorine** for a **contact time of 10 minutes** is acceptable as stated.

3. In the previous DER dated November 15, 2005, the Agency requested that the registrant submit efficacy data for the non-public, non-OIE animal pathogens Canine Distemper virus, Infectious Canine Hepatitis, African swine fever, Porcine parvovirus, Runting & Stunging virus (tenysynovitis, **correct spelling "tenosynovitis"**), Actinobacillus pleuoropheumonia (**correct spelling "pleuropneumoniae"**), Bordetella bronchiseptica (rhinitis), Brachispira **(correct spelling "Brachyspira)** (Treponema/Serpuline) hyodysenteriae (swine dysentery), *Streptococcus uberis*, *Streptococcus dysgalactiae*, and *Clostridium perfringes* USDA. Submission of the requested efficacy data is no longer needed by the Agency.